TÍTULO DA TESE: Characterization of the subtilisin correlated with rubisco proteolysis

Abstract

A subtilisin was identified, characterized and isolated from wheat leaves. *In vitro*, it has the ability to degrade ribulose bisphosphate carboxylase (EC4.1.1.39; rubisco), showing enhanced proteolytic activity towards the oxidized form of the enzyme. During nitrogen starvation, the presence of the active form is confirmed by detection of its autocatalytic products, which are correlated with rubisco degradation. Rubisco proteolysis is detected in both the basal and the tip segments of the stressed wheat leaf blade, which indicates independence from senescence progression. This subtilisin is located in the vicinity of chloroplasts, probably inside vesicles.

Plant subtilisins are abundant proteases that exhibit broad substrate specificity. However, they may be induced in particular developmental stages, where they perform specific tasks.

Stress conditions or senescence can lead to the formation of numerous vesicles-containing proteases. Analysis of *Arabidopsis thaliana* transcriptome reveals that the subtilisin under study is preferentially expressed in leaves, and its expression is enhanced during senescence. The cysteine protease RD21A correlates with this subtilisin, both at transcriptional level and cellular localization, suggesting that both proteases participate in rubisco degradation.

The results suggest that the degradation of rubisco may occur outside the chloroplasts in the senescence associated vesicles.

KEY-WORDS: proteolysis, rubisco, senescence, stress, subtilisin.